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*Vector Biology
and Control Project*

**Assessment of Insecticide Safe-Use
Practice and Insecticide Resistance
Testing Procedures in the Pakistan
Malaria Control Program**

September 9-30, 1990

by

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Acronyms and Abbreviations

A.I.D.	Agency for International Development
API	Annual Parasite Incidence
DOMC	Directorate of Malaria Control
EA	Environmental Assessment
ER	External Review
GOP	Government of Pakistan
HPN	Health, Population and Nutrition (Division, USAID/Islamabad)
IPM	Integrated Pest Management
MCP	Malaria Control Program (of Pakistan)
MCP II	Malaria Control Project II Extension
NIMRT	National Institute of Malaria Research and Training
NMCP	National Malaria Control Program
NWFP	North West Frontier Province
OP	Organophosphate
PCD	Passive Case Detection
SPR	Slide Positivity Rate
ULV	Ultra-Low-Volume
UNHCR	United Nations High Commission for Refugees
USAID	United States Agency for International Development
VBC	Vector Biology and Control Project
WHO	World Health Organization
WDP	Wettable Dispersible Powder

1. Executive Summary

The Malaria Control Project II (MCP II) requested technical assistance in evaluating the 1990 spray operations in Pakistan to determine whether safety procedures were followed and assess the reliability of WHO insecticide resistance tests as carried out by provincial and district entomological staff of the Directory of Malaria Control (DOMC). The project paper for MCP II as authorized by USAID/Islamabad, the DOMC and the Government of Pakistan (GOP) contains specific conditions for pesticide safe-use and specifies that annual assurances be made that the conditions are being met.¹ The USAID/Singapore auditor in Audit Report No. 5-391-90-05 (January 31, 1990) requested an assessment of the reliability of the WHO insecticide resistance test data as performed by DOMC staff.²

To accomplish these objectives, USAID/Islamabad and GOP prepared a scope of work and asked A.I.D.'s Office of Health to provide consultancy services to meet the scope of work. I was engaged by Vector Biology and Control Project (VBC) to carry out the job. This consultation began with a background briefing in Washington, D.C. I arrived in Pakistan on September 9, 1990, and received briefings in Islamabad before visiting all four provinces of Pakistan to assess insecticide safe-use practices and insecticide-resistance test procedures. I also reviewed reports of previous consultants and other relevant data on the Malaria Control Program of Pakistan (MCP).

This report contains findings and recommendations based on field visits to DOMC operational units in all four provinces. Correct procedures, deficiencies, constraints and recommendations are enumerated.

I found that the way malaria workers used insecticides varied greatly from province to province as well as within each province. In general, the insecticide malathion is being used safely so that it doesn't intoxicate spraymen or mixers or harm the environment. There were safety lapses that, if left uncorrected, could harm applicators and the environment. Basically the WHO insecticide-

resistance testing is being carried out satisfactorily, and the test results are reliable; however, three of the four provinces carry out too few tests.

I recommend that the current guidelines of both Evaluation Format MCP II (Annex 4) and MCP (Annex 5) on safe-use of the insecticide malathion be strictly adhered to as well as strengthened. This is necessary to prevent possible human intoxication and environmental damage. Additional training, strengthened supervision and closer monitoring for compliance with all safe-use procedures are also recommended.

WHO testing should be more precisely and extensively carried out. Further, the MPC's entomology staff should receive intensified training. Job requirements for entomologists could be upgraded. Moreover, additional entomological techniques should be used to complement and supplement the basic WHO adult resistance tests.

As carried out by the DOMC, the MCP is basically a good program that is controlling malaria in Pakistan. It can, however, be refined and adjusted. My findings, comments, suggestions and recommendations are meant to be constructive, rather than critical.

2. Introduction

Purpose

This activity was intended to assess insecticide safe-use practices as required by the scope of work prepared by USAID/Islamabad and the Project Paper for MCP II Project Extension.¹ It was also to determine the reliability of procedures that test for insecticide-resistance, as required by USAID/Islamabad to meet the recommendations of the USAID/Singapore auditors.²

Scope of work

The consultancy's scope of work was as follows:

1. To help the project officer and director of the Directorate of Malaria Control (DOMC), government of Pakistan, evaluate 1990 spray operations in all four provinces.
2. To assess whether spray operations meet safety procedures taught in annual training programs for malaria control staff.
3. To assess the reliability of mosquito insecticide-resistance tests carried out by the provincial and district entomological staff during spray operations.
4. To prepare a consultancy report summarizing observations, analysis and recommendations on objectives 1 and 2 above in all four provinces and submit it to USAID/Islamabad before departure.

Planning

On arriving in Pakistan, I met with Ms. Anne Aarnes, chief of the Office of Health, Population and Nutrition (HPN), USAID/

Islamabad; Dr. Rifaq Ismail, project officer, MCP II, USAID/ Islamabad; and Mr. Chaudhary A. A. Mujahid, director, DOMC, Islamabad, for briefings. Then Dr. Rifaq arranged a travel itinerary with the cooperation of Mr. Mujahid, provincial MCP staff and USAID liaison officers, to accomplish the two main objectives of the consultation.

Methods

I personally observed malathion spray operations in all four provinces during my visit from September 9-30, 1990. I observed WHO insecticide susceptibility tests in two provinces and reviewed all available WHO test data from the other two provinces. Also, I observed cholinesterase testing in one province and reviewed available records in the other provinces.

Background

The GOP has carried out nationwide malaria control program consisting of indoor spraying of residual insecticide more or less continuously since 1961. The principal malaria vector throughout the country is *Anopheles culicifacies*. The secondary vector is *Anopheles stephensi*. After *An. culicifacies* developed resistance to DDT, dieldrin and BHC, which were used as insecticides early in the program, all provinces switched to malathion in 1976. Malathion as a 50 percent wettable dispersible powder (WDP) is applied to interior walls and roofs at a dosage of two grams active ingredient per square meter. In 1980, *An. stephensi* began to show signs of tolerance to malathion. By 1982, this tolerance had developed into resistance; 20 percent or more of the *An. stephensi* targeted survived the WHO tests with malathion-impregnated test papers. Somewhat later, tolerance and slight resistance to malathion, as measured by the WHO tests, began to appear in *An. culicifacies*. Malathion resistance in *An. stephensi* has reached high levels in 18 districts in the country; 14 of these districts are in Punjab province (Annex 3).

Malathion tolerance in *An. stephensi* first appeared in Punjab in 1980,³ followed by its appearance in the province of NWFP in 1982, and in Sindh and Baluchistan in 1984. *An. culicifacies* has developed malathion tolerance much slower. Only in two districts in Punjab province (Vehari and Jhang) has resistance to malathion developed in *An. culicifacies* (Table 1).

The MCP has emphasized insecticide safe-use practices since the inception of malaria control in 1961. Annual training sessions for both supervisors spraymen and mixers, however, have been regularly carried out only since 1983. Safety supplies have also been issued to each sprayman and mixer by the GOP.

During August 13 - September 4, 1989, Dr. Jack Hayes visited Pakistan as a VBC consultant and prepared a report "Evaluation of the Safe-Use of Malathion in the Pakistan malaria Control II Project."⁴

In his report, Dr. Hayes expressed certain concerns over safe use of malathion, field training practices, storage of malathion, and compliance by DOMC with recommendations in the Environmental Assessment (EA) prepared by Drs. Jesse Hobbs and Hiram Larew (July 31 - August 20, 1988)⁵. Dr. Hayes visited two provinces: Punjab and NWFP. I also reviewed "Report of an External Review of the Pakistan Malaria Control Project,"⁶ prepared by a team of malaria specialists from VBC, the WHO and the GOP in March 1990.

Table 1
Consolidated Record of Susceptibility for the Year 1989,
Punjab Province

S. No.	Name of District	INSECTICIDES								INSECTICIDES								Total															
		D.D.T. 4.0%				D.L.D. 4.0%				Malathion 5.0%				Fenitrothion 1.0%																			
		Culicifacies		Stephensi		Culicifacies		Stephensi		Culicifacies		Stephensi		Culicifacies		Stephensi																	
		Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.																
1.	Lahore	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	3	3	0	0	2	1	0	0	0	-	8				
2.	Kasur	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	1	4	6	6	0	0	6	12	0	0	12	-	29				
3.	Sheikhupura	5	0	0	0	0	0	0	0	4	4	0	0	0	11	0	0	11	3	3	0	0	10	0	0	0	0	-	33				
4.	Ukara	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	14	14	0	0	0	10	0	0	10	-	25				
5.	Gujranwala	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	7	0	0	0	0	2	0	0	2	0	0	0	-	9		
6.	Gujrat	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	1	9	2	1	1	0	8	0	1	7	1	0	0	1	-	20	
7.	Sialkot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	0	0	0	-	2	
8.	Rawalpindi	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	2	1	1	0	0	0	0	0	0	0	0	0	-	4	
9.	Attock	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2	0	0	2	0	0	0	0	0	0	0	0	-	3	
10.	Jhelum	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	3	0	0	3	0	0	0	0	0	0	0	0	-	4	
11.	Chakwal	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	4	0	0	4	0	0	0	0	2	0	0	2	-	7	
12.	Sargodha	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	3	3	3	0	0	3	0	0	3	2	0	0	2	-	11	
13.	Khushab	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	3	3	0	0	0	4	0	0	0	2	0	0	2	-	6
14.	Mianwali	1	1	0	0	0	0	0	0	1	0	1	0	0	3	0	0	3	6	2	3	1	0	0	0	4	2	0	0	2	-	17	
15.	Bhakkar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
16.	Faisalabad	3	3	0	0	5	5	0	0	2	1	1	0	5	6	0	1	5	9	8	1	0	6	0	0	6	8	0	0	8	1*	44	

R= Resistance
(80% or less
mortality)

T= Tolerant
(80% to 98%
mortality)

S= Susceptible
(98% to 100%
mortality)

(*) Progeny tests with stephensi indicated increased resistance in all 6 F₁ tests.

Table 1

**Consolidated Record of Susceptibility for the Year 1989,
Punjab Province, (continued)**

S. No.	Name of District	INSECTICIDES								INSECTICIDES								Total																		
		D.D.T. 4.0%				D.L.D. 4.0%				Malathion 5.0%				Fenitrothion 1.0%																						
		Culicifacies		Stephensi		Culicifacies		Stephensi		Culicifacies		Stephensi		Culicifacies		Stephensi																				
		Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.	Tot:	R. T. S.																			
17.	Toba Tek Singh	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	-	3												
18.	Jhang	3	3	0	0	0	0	0	0	5	5	0	0	1	1	0	0	18	11	7	0	16	16	0	0	15	0	5	10	10	0	9	1	-	68	
19.	Multan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	15	7	4	0	3	8	0	0	8	0	0	0	0	-	30	
20.	Khanewal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	3	3	2	0	1	2	0	0	2	1	0	0	1	-	9	
21.	Vehari	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	2	3	5	8	7	1	0	5	1	2	2	4	0	0	4	-	27	
22.	Sahiwal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	26	26	0	0	0	0	0	0	0	14	0	0	14	-	41
23.	Bahawalpur	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0	17	5	8	6	2	0	21	0	0	21	7	0	0	7	-	58	
24.	Bahawal Nagar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	3	11	11	0	0	3	0	0	3	11	0	0	11	-	28	
25.	Rahim Yar Khan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	1	0	0	0	0	0	0	4	0	0	4	-	8
26.	Dera Ghazi Khan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	4	4	25	25	0	0	6	0	0	6	16	0	0	16	5*	55	
27.	Rajapur	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	-	1	
28.	Muzaffargarh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	10	4	6	0	0	0	0	0	7	0	0	7	-	19	
29.	Layyah	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GRAND TOTAL		12	12	0	0	5	5	0	0	12	10	2	0	6	5	1	0	136	13	37	86	181	148	16	17	103	1	8	94	114	0	9	105	6*	569	

R= Resistance
(80% or less
mortality)

T= Tolerant
(80% to 98%
mortality)

S= Susceptible
(98% to 100%
mortality)

(*) Progeny tests with stephensi indicated increased resistance in all 6 F₁ tests.

3. Findings

Insecticide safe-use

North-West Frontier Province (NWFP)

Intradomiciliary spraying with 50 percent malathion WDP was observed in the village of Jatti Payai (Peshawar district). This was a typical Pathan village with houses made of katcha (mud/straw walls) with wood/grass roofs. I watched the entire process of mixing the insecticide, filling the Hudson x-pert sprayers, preparing the room and actually spraying rooms. Proper procedures and safety precautions were observed. I did not see empty malathion boxes burned but was told by DOMC personnel present that they would be. I also watched malathion spraying operations at Gujar Glari (Mardan district). All safety procedures were followed except that the mixer had leather gloves instead of rubber gloves, which could expose the mixer to more insecticide if the gloves became wet with insecticide.

I observed a storage building for holding malathion in the Mardan District. The building was constructed suitably. It was separate from other buildings, and it was locked. The insecticide was protected from the weather and an inventory card was present.

The MCP officer assured me that cholinesterase tests are conducted on all spraymen and mixers twice: before spraying begins and two to three weeks later. A appropriate supply of atropine sulphate ampules and sterile syringes were present at both of the spray sites described above. Each sprayman and mixer had two sets of uniforms, and soap was available. I asked if any sprayman or mixer had become intoxicated by the spraying or mixing; I was told no.

Cholinesterase data were recorded for each sprayman and such data had been kept for several years. MCP officers indicated that a one-week meeting on insecticide safety is held for all supervi-

sory personnel each year before the spray season. The spraymen and mixers are also trained in the field for at least one day before they start spraying. A trained supervisor closely watches each spray squad of six men (five spraymen and one mixer) during the spray operations.

Punjab

I witnessed malathion spray operations in the village of Kuleki (an approximate population of 1,200, 120 houses), Union Council Lakhoki, Lahore district. All safety procedures were followed. I observed the malathion storage at a rural health clinic at Arki. The insecticide was stored in a separate building at least 30 meters from any other building. The building was elevated, locked and rain-tight with ventilation, and an inventory card was present. I also observed focal spraying conducted in the village of Haveli Ajaib Singh because of two cases of *P. falciparum* malaria. Spray operations were performed satisfactorily and safely.

In Haveli Ajaib Singh, cholinesterase testing was performed on all spraymen and mixers. The procedure of tintometric testing was carried out properly. One sprayman had a cholinesterase level of 75 percent (indicating 25 percent depression), which indicates excessive exposure to the insecticide. The sprayman was given two days off and warned to be more careful in spraying when he returned. Standard procedure, which is ultrasafe, calls for two weeks off; however, MCP officers present said they could not afford to have the worker off for two weeks.

In Punjab province, supervisors and officers are given safety training for one day before the spraying season starts. This year, training was held in Faisalabad. Spraymen and mixers are trained for two or three days.

In general, from the beginning to the end of contact with malathion, the insecticide was being handled safely and properly in Punjab. The exception is that the malathion containers are kept after use, brought back to a central location and sold to a local contractor who buys, cleans and reuses the boxes. The

contractor agrees not to ship foodstuffs in the recycled boxes. Funds collected from this procedure go to the provincial government, not the DOMC. This procedure should be discontinued.

Sindh

I watched spray operations in Wahid Dino Shoro Village (Thatta District). Spraying started in Thatta District on September 22 and was scheduled to end about October 15. In Thatta, the spray squad consisted of four spraymen and one mixer.

Spray operations, including mixing and actual spraying, were fine. A disposal pit was dug so that the wash water of mix buckets and spray tanks could be disposed of safely. Sindh was the only province in which I observed this procedure. MCP officers told me that all boxes are burned after use and that cholinesterase tests are given to all spraymen and mixers before and during the spray season.

I also watched spray operations in Gulhasan Sarawol Village (Sanghar District) and inspected Zahidabad Village (Sanghar District) immediately after spraying to observe completed spray operations. Observations in both instances were satisfactory. Spraymen in Sanghar District started spraying on August 10 and should have finished by October 7. Supervisory personnel are trained each year, and spraymen and mixers are trained for two days.

Baluchistan

I observed spraying of the Pathan village of Kharotabad on the outskirts of Quetta. Four spray squads were rapidly completing this large katcha-walled village. All spraying procedures were satisfactory except in one instance where a worker was mixing insecticide without gloves, although he had gloves nearby. Dr. Fazal Ahmed, USAID liaison officer, who also helps evaluate malaria spray operations for MCP II, observed spraying operations in Bela District the day before I arrived in Quetta. Dr. Fazal said that he saw a mixer mixing malathion with his bare hands without gloves or a paddle; in addition, the mixer had only

one uniform and two buckets instead of the required three. These are, of course, very serious violations of good safety procedures and could result in intoxication. Obviously there are training and supervision deficiencies in this instance that should be immediately rectified. Dr. Fazal reported his findings to the provincial chief in Quetta the next day.

Spraying started in Quetta Zone on August 21 and was scheduled to end September 21. All spraymen and mixers are given three cholinesterase tests each season. I was told that spraymen and mixers are given three days practical training.

I inspected the main insecticide storage facility for Baluchistan province, which is located in Quetta. This storage depot was grossly deficient in several aspects. One hundred forty boxes of malathion 50 percent WDP were stored outside on the ground with only a short piece of visqueen covering them. There were 20 to 30 empty malathion boxes being stored outside with no cover. In a nearby building, 425 boxes of malathion and 110 (5-gal.) drums of Abate EC were stored. Another problem is that the caretaker and his family live next door to the insecticide storage room and share a common roof. There is a real possibility of airborne malathion particles spreading into the caretaker's home. This situation should be rectified immediately, and all empty malathion boxes should be burned in an open area. DOMC officers were apprised of this situation.

General Overview

Spraying is being carried out in all provinces in a generally satisfactory manner. I saw isolated instances (in Sindh and Baluchistan) of breakdowns of safe-use practices, as pointed out above. A wide range of handling procedures are being followed in each province however. Training of spraymen and mixers varies greatly from province to province. For example, in some provinces supervisors are trained for one day, and in other provinces, for five days. Spraymen are trained for one day in some provinces and for three days in others. Supervisors and spraymen should be trained regularly and conscientiously. Safety and safe-use of insecticides require continuous vigilance.

Spraymen and mixers are paid differently in the provinces. For example, in Punjab they are paid 35 to 52 rupees per day; in Sindh, 30 rupees per day; in NWFP, 25 rupees per day, and in Baluchistan, 38 authorized, (50 rupees per day actually received). The age of spraymen and mixers ranges from 15 to 60-plus years. Older spraymen and mixers appeared to be doing a better job.

The disposal of used malathion boxes varies greatly from province to province. In Punjab, they are collected and sold. In some provinces, they are burned, and in others, saved and stored. The instructions on all malathion boxes that I saw in Pakistan were in English only. In the future, instructions should also be in Urdu, the national language of Pakistan.

The MCP's spray evaluation sheet (modified 1990) is excellent (Annex 4). Its use should be encouraged, and all spray squads in the country should be evaluated at least once each season, preferable early in the spray cycle, by district or province DOMC officers. Further, the evaluator should act on the evaluation information to rectify any deficiencies or infractions. Serious or gross problems should be referred to officers or the district health officer and even the Provincial Chief, if necessary. Spray squads that are in serious violation should be reevaluated by a different evaluator within one week of the first negative evaluation. If violations are still apparent at the second evaluation, remedial action should be taken, even to the extent of dismissing the responsible personnel.

Each province prepares an annual instruction manual spray operation for (Annex 5). This instruction is very good, and it covers important safety aspects of malathion use. If all spray squads follow instruction rigidly, there will be few problems with insecticide safety.

Recommendations

1. Training on insecticide safe-use should be emphasized and all supervisors and spraymen and mixers should have to pass a practical field test after training. When any supervisor, sprayman or mixer fails to display good insecticide-handling practices, he should be dismissed or, in the case of spraymen and mixers, not rehired.
2. Spraymen and mixers should be paid more so that better personnel can be recruited, hired and retained, preferably from season to season.
3. Insecticides must be respected. The more training and education people have, the more they respect insecticides.
4. Disposing of all malathion boxes by burning them in an open, safe manner should be mandatory in all provinces. The small amount of money recovered from selling the used cartons is not worth the risk of insecticide intoxication, accidental misuse, and giving the public the wrong impression about insecticide safety.

Insecticide Resistance Testing

NWFP

I observed the collection of *Anopheles* specimens from an animal shelter in a typical Pathan katcha-walled compound. Specimens of *An. stephensi*, *An. culicifacies*, *An. subpictus* and *An. fluviatilis* were collected by aspiration. Although there were not enough specimens for WHO testing (a minimum of 50 of one species would have been needed: 25 for the test and 25 for the control), the district entomologist ran through the entire test procedure, and his technique and knowledge of procedures were very good. I reviewed the latest WHO test data from the

province with the entomologist. After observing the collection, handling, and trial run of the WHO insecticide susceptibility test, I think the data are accurate and reliable. There is resistance to malathion in certain *A. stephensi* populations in NWFP.³

Punjab

Two separate WHO tests were run from start (collection) to finish in the Lahore and Kasur districts. Seventy-five *An. stephensi* were collected (enough for two tests and one control) in Khano Hurni Village, and 75 *An. stephensi* were collected in Patto Kalan Village (Kasur District) for another test. The results of these tests are presented on page 14 (Table 2).

The results were not surprising. MCP entomologists had detected high levels of resistance to malathion in *An. stephensi* in these districts. Fortunately, the primary vector of malaria in Punjab province, *An. culicifacies*, is still susceptible to malathion except in two districts of the province (see Table 1). Both species *stephensi* and *culicifacies* are still basically susceptible to fenitrothion, another organophosphate (OP) insecticide that WHO has approved for residual house spraying.

Punjab province has an abundance of WHO susceptibility test data. Dr. Georghiou summarizes these data in his 1990 report.³ Punjab contains more than 50 percent of Pakistan's population in only 26 percent of the country's land area. The province is the agricultural center of Pakistan. It has an abundance of water and many *Anopheles* breeding sites. Therefore, the province does the most spraying for malaria control in the country. In addition, the agricultural sector uses the most insecticide of any province. It is not surprising, with the intense insecticide selection pressure from agricultural spraying and malaria control that resistance would appear here first and be more widespread and more intense.

**Table 2. Results of WHO Susceptibility Test with
An. stephensi in Punjab Province, 1990**

September
17, 1991

District	Locality	U/Council		Dead	Total	Mortality
Lahore	Khano Hunri	Julke	Fenitrothion 1 %	25	25	100%
			Malathion 5 %	17	25	68%
			Control	0	25	0.0%

September
18, 1990

District	Locality	U/Council		Dead	Total	Mortality
Kasur	Pattokalan	Pattokalan	Fenitrothion 1 %	25	25	100%
			Malathion 5 %	18	25	72%
			Control	1	25	4%

Sindh

When I visited Sindh province, no entomologists or assistant entomologists were available to collect vectors and do WHO testing. I did, however, obtain the latest WHO test data from the province and discussed the testing with the province's senior malaria superintendent.

Baluchistan

The province has only one experienced entomologist, and he was in Lahore for training at The National Institute of Malaria Research and Training (NIMRT). I reviewed the latest 1990 WHO test data with MCP officers in Quetta. These data are presented in Table 3. Baluchistan is the opposite of Punjab: it has the largest land area of any province (43 percent) and the smallest population. I examined the WHO test kit and found everything in order, including unexpired test papers.

Table 3. Vector Susceptibility Based on WHO tests with Malathion 5%*
Records for the year 1990 through 9/20/1990,

District	Sub. Section	Locality	Date	Species	Spray History	Total Exposed	Total Dead	(%) Mortality
Loralai	a-3	Patan Kot	4/24/90	Steph.	Spray with Malathion in Jul. 89	45	20	44.4
Loralai	a-3	Pathan Kot	4/27/90	Steph.	-do-	25	13	52.0
Quetta	a-2	Subzal	5/15/90	Steph.	-do-	30	23	76.6
Quetta	a-2	Kuchlak	5/16/90	Steph.	-do-	25	14	56
Pishin	b-3	Muchan	5/20/90	Cul.	-do-	28	27	96.42
Pishin	a-8	Bostan	5/21/90	Cul.	-do-	28	28	100
Kalata	a-6	Mastung	5/25/90	Cul.	-do-	38	29	96.66
Kalat	a-2	Surghaz	5/25/90	Cul.	-do-	25	25	100
Sibi	a-2	Tali	5/28/90	Steph.	Sept. 89	225	20	80
Sibi	a-6	Khajak	5/29/90	Cul.	-do-	25	25	100
Quetta	a-2	Subzal	6/02/90	Steph.	Nil	29	11	37.93

(1) Data supplied by Provincial Chief

(*) Exposure Period 1 Hour

**Table 3. Vector Susceptibility Based on WHO tests with Malathion 5% *
Records for the year 1990 through 9/20/1990₁, (continued)**

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District	Sub. Section	Locality	Date	Species	Spray History	Total Exposed	Total Dead	(%) Mortality
Pishin	b-3	Muchan	6/04/90	Culi	Sept. 89	26	26	100
Sibi	a-2	Tali	7/12/90	Culi	-do-	24	24	100
Jafarabad	b-2	Got Zahoor	7/13/90	Culi	Nil	23	23	100
Jafarabad	a-3	Got Raza M.	7/13/90	Steph.	-do-	24	20	83.3
Tumbo	a-1	Murad Jamali	7/14/90	Culi	Sept. 89	30	29	96.66
Tumbo	a-1	Murad Jamali	7/14/90	Steph.	-do-	28	24	85
Quetta	a-2	Subzal	7/20/90	Steph.	Nil	25	20	80
Quetta	a-2	Kharot Abad	7/25/90	Steph.	July 89	26	21	80.76
Pishin	a-8	Bostan	7/28/90	Culi	-do-	22	22	100
Pishin	b-3	Muchan	7/29/90	Culi	-do-	24	24	100
Kalat	a-6	Mastung	7/30/90	Culi	-do-	30	29	96.66
Lora Lai	a-3	Patan Kot	8/12/90	Steph.	Aug. 89	23	10	52
Lora Lai	a-3	Patan Kot	8/12/90	Steph.	-do-	25	15	60
Quetta	a-2	Subzal	8/13/90	Steph.	Nil	28	18	64.28
Quetta	a-2	Kuchlak	8/15/90	Culi	Aug. 89	25	25	100
Pishin	b-3	Muchan	8/18/90	Culi	July 89	29	28	96.55
Kalat	a-6	Mastung	8/20/90	Culi	-do-	24	24	100

(1) Data supplied by Provincial Chief

(*) Exposure Period 1 Hour

Overview

I feel the WHO insecticide susceptibility test data are reliable. After examining extensive reports and observing first-hand the test procedures in two provinces, I found only minor deficiencies and omissions, such as relative humidity or temperature data not recorded or too few mosquitoes tested for valid results (e.g. 16 or 18 versus 25 per tube). Control mortality is low, so test mosquitoes are handled adequately. Test papers are current; I found none that had expired. WHO now makes organophosphate test papers that last three years for insecticides and five years for controls.

Adequate testing with WHO kits is conducted in Punjab province; however, more tests should be conducted in the other three provinces. Additional entomological data on vector species could be very beneficial to control efforts.

The disturbing observations from the WHO resistance testing in Punjab province are that *An. culicifacies* has developed resistance to malathion in two districts (Vehari and Jhang) and that fenitrothion seems to select for resistance in *An. culicifacies* stronger and faster than *A. stephensi* (Table 1). This latter observation needs to be studied in greater detail. Such "pre-resistance" in *An. culicifacies* could preclude the use of fenitrothion to replace malathion. Also, resistance in *An. culicifacies* to both malathion and fenitrothion is an ominous sign because malaria control officers' in Punjab province consider this species the principal vector of malaria there. Both the malathion and fenitrothion resistance in *An. culicifacies* and *An. stephensi* in the Vehari and Jhang districts are almost certainly related to intense and widescale insecticide use in agriculture.

Recommendations

I recommend that more WHO susceptibility testing be done in NWFP, Sindh and Baluchistan at all fixed locations, once before the spray season each year and once after. Random tests should be conducted to detect incipient resistance in areas where malaria transmission is high, where *P. falciparum* cases predominate or

where there are unusual changes in the vector ratio of *An. stephensi* to *An. culicifacies*. I also recommend that additional entomological techniques, such as bioassays, larval surveys, larval WHO susceptibility tests and adult collections, be carried out to better understand vector bionomics. The more that is known about vector bionomics, the more skillfully can alternative control measures be applied, such as larviciding, source reduction, biological control and even use of synergistic insecticides.

Intensified and expanded entomological training for all entomologists (including assistant entomologists) is strongly recommended. Consideration should also be given to drawing up more specific job descriptions for both entomologists and assistant entomologists, which may help improve salary structure. Training and instruction should emphasize field methods. I also recommend that practical field tests be given to all trainees. All entomologists in the country should be able to conduct WHO tests and bioassays, collect vectors, and differentiate and collect *Anopheles* larvae.

The research capabilities of NIMRT and DOMC should be strengthened through training, personnel acquisition, technical assistance, and the acquisition of new equipment and supplies. NIMRT research could be oriented more toward the long term and deal with more sophisticated methodologies, such as ELISA, sibling species determination, genetic research, bloodmeal precipitin testing, vector competence and other research that requires expensive equipment and carefully controlled conditions. DOMC research, on the other hand, could be field research that is operationally oriented, such as breeding-site reconnaissance, larval and adult identification, biological control, biting collections, light trap collections and larvicide testing. NIMRT research should complement that of the MCP.

Conducting practical training courses in the field in the individual provinces rather than in Lahore or Islamabad is highly desirable for several reasons. It would be easier and more

economical for two trainers or instructors to go to Sindh than to bring 15 trainees to Lahore. Also, the local vector problems of Sindh, Baluchistan and NWFP are greatly different from those in Lahore.

I heard complaints from MCP personnel in Sindh and Baluchistan province that entomologists do not have transportation to far-flung locations to do entomological work. I could not tell whether this complaint was valid. If it is, more transportation for entomologists should be considered.

4. References

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Annex 1. People Consulted

September 9-30, 1990

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Annex 2. High-Resistance Districts

Ranking of High-Resistance Districts of Punjab, Sindh and NWFP, and Areas under the Control of the U.N. High Commission for Refugees (UNHCR), in Decreasing Order of Resistance to Malathion Observed in *An. stephensi* during the Latest Two Years of Tests (1988 - 1989)

District	Province	Average Mortality % WHO Test
Kohat	UNHCR	18.7
D.G. Khan	Punjab	26.6
Rajanpur	UNHCR	28.6
Bahawalnager	Punjab	29.0
Y. Gh. Mohamand	UNHCR	31.5
Sanghar	Sindh	34.5
Bahawalnagar	Punjab	35.5
Rawalpindi	Punjab	41.6
Okara	Punjab	42.5
Sukkur	Sindh	43.1
Khushab	Punjab	43.6
Lahore	Punjab	44.4
Kasur	Punjab	45.7
Shiekhupura	Punjab	52.9
Sahiwal	Punjab	53.6
Faisalabad	Punjab	53.7
Sargodha	Punjab	53.9

From Georghiou, G. P., 1990³

Annex 3

Spray Evaluation Form

Annex 4

Instruction for Spray Operation 1990 (Sindh)

1. Spray campaign will be started in 1st week of August 1990 depending upon the availability of transport and distribution of insecticide to planned positive localities.
2. Villages/localities under planned areas will be sprayed as per criteria already communicated via this Headquarters letter No. OPR/SPR/1990/4773/86/88, dated 24th December, 1989.
3. Dose of malathion 50% W.D.P. will be 2 gram per square metre.
4. The spray operation will be completed in 45-50 working days within the allocated amount for seasonal labours.
5. Every laborer will cover at lease 30-35 rooms per day.
6. Recruitment of the spraymen will be at sector Headquarters by the board of officers as authorized by the District Health Officer. Preferably the sector in charge of the area will also be included in the board. Any change in spraymen should be brought to the notice of District Health Officer.
7. Cholinesterase level of each sprayman will be done at the time of recruitment, as well as during spray operation. The proper record of the test performed will be maintained at the district level and a copy of the same will be sent to this Headquarters.
8. Training in spray technique/safe-use of malathion will be imparted to malaria supervisors/spraymen before the start of spray operation.
9. In case any early symptom of poisoning occurs, the supervisors will properly look after the patient as they learned in the training of safe-use of malathion, and will seek necessary advice of

a medical officer/doctor available as near as possible. The Assistant Malaria Superintendent will be immediately informed of who will be responsible for case follow-up. All serious cases should be brought to the personal attention of the District Health Officer concerned. The Provincial Chief should be informed by telegraph in case of any serious poisoning. All expenditure made in the treatment of patient should be borne by the department. Atropine and syringes should be made available to all supervisors for immediate treatment on appearance of any toxic symptom.

10. The insecticide should be stored properly in villages under safe custody to avoid leakage and toxic hazards.
11. ASO will remain in progress during the spraying operation.
12. Mixing stations for insecticide should be 5 metres away from the sources of useable water supply.
13. All workers must take a bath after daily work.
14. All clothes and spraying equipments must be washed daily (inside and outside) after finishing the work.
15. All mixers must have a wooden stick sufficiently long to mix the insecticide and their hands must be protected by gloves.
16. Supervisor should see personally that no insecticide leaks from the sprayers to contaminate the body of spraymen.
17. In case of omission/missing of any positive locality/localities may be included in the spray plan on the basis of epidemiology data under intimation to this Headquarters.
18. All instructions on safe-use of malathion explained during training must be followed strictly.

(Dr. Ghulam Rasool Shaikh)
Provincial Chief

Annex 5

General Observations on MCP

(These are personal suggestions of Mr. Stokes and are not necessarily supported by the VBC Project.)

1. Malathion house spraying as currently carried out at a rate of two grams per meter and one application per year is effective throughout all four provinces of Pakistan. This is true in spite of high levels of malathion resistance in localized populations of *An. stephensi*.

Certain localities within 14 districts in Punjab province that Georghiou cited as having highest resistant populations of *An. stephensi* show API and SPR declines from 1989 to 1990; moreover, annual parasite incidences (APIs) and slide positivity rates (SPRs) are lower than in other areas of the province with no resistance (Table 4).

2. In certain localities in all provinces where little or no insecticide selective pressure has been exerted, the vector species *An. stephensi* is 100 percent susceptible to malathion. To preserve malathion as an effective residual insecticide, the MCP should consider rearing or collecting large numbers of susceptible males and releasing them in localities where resistance is worst. Such gene replacement or gene dilution might restore susceptibility. To enhance this strategy, a small, isolated locality with high resistance should be chosen, and larviciding source reduction methods could be used to reduce the target (resistant) population so that fewer susceptible males would be required. Comparative costs should be based on the additional cost of switching to fenitrothion from malathion.

Table 4

**Ranking of 14 High Resistance Districts of the Punjab
of *An. stephensi* to Malathion**

S. No.	Districts	1988		1990		Average Mortality (%) 1988 - 1989 WHO Tests***
		SPR*	API**	SPR*	API**	
1.	D. G. Khan	3.23	2.07	3.34	0.86	26.6
2.	Rajanpur	2.14	1.01	2.14	0.80	28.6
3.	Bahawalpur	1.98	1.10	1.35	0.40	29.0
4.	Bahawalnagar	0.66	0.36	0.20	0.06	35.5
5.	Rawalpindi	1.12	0.71	0.51	0.17	41.6
6.	Okara	1.33	0.71	0.18	0.07	42.5
7.	Khushab	2.36	1.12	0.58	0.16	43.6
8.	Lahore	0.57	0.24	0.44	0.08	44.4
9.	Jhang	2.32	1.04	1.23	0.32	45.7
10.	Kasur	0.37	0.21	0.31	0.08	46.6
11.	Sheikhupura	5.22	1.84	1.60	0.22	52.9
12.	Sahiwal	0.41	0.20	0.29	0.04	53.6
13.	Faisalabad	1.14	0.37	0.31	0.04	53.7
14.	Saragodha	3.42	1.77	1.77	0.36	53.9

* January 1 - December 31, 1989

** January 1 - July 31, 1990

*** From Georgiou, 1990

3. Using synergistic residual adulticides should also be considered in an effort to forestall further development of resistance (for example, malathion and permethrin, or even DDT and malathion). Two to three insecticides of different chemical classes (such as OP, carbamate or pyrethroid) could be rotated yearly so that resistance against one chemical does not have a chance to develop. Further consideration might be given to testing negatively correlated insecticides with different detoxification routes in the vector species.
4. The cities (municipal corporations) of Pakistan and the MCP do not coordinate their malaria control work. At present, the cities are doing their own thing, using highly toxic larvicides such as fenthion and doing ultra-low-volume (ULV) spraying with various insecticides, often without adequate supervision and entomological backup. This practice could exacerbate the resistance problem and could result in potential safety problems to applicators and the environment. It would be better for the cities and the MCP to plan jointly and for MCP and DOMC to have oversight over all mosquito control activities.
5. The Afghan refugee camp mosquito control efforts are essentially independent of each other (camp to camp) and from that of the MCP. The MCP should jointly plan, coordinate and maintain oversight over all mosquito control efforts within refugee camps.
6. Virtually all of Baluchistan and parts of Sindh lend themselves to source reduction, larviciding and biological control techniques because of their arid climate and isolated malaria vector breeding sites. The larger districts in Pakistan, such as Peshawar, Quetta, Karachi and Lahore, lend themselves to alternative control measures by virtue of the kinds and lesser magnitude of vector breeding sources within their confines.

Source reduction and other alternative control measures are the only way to deal with a sizable migrating nomadic population that lives in tents or open shelters, such as exist in Baluchistan and Sindh. These nomads do not seek medical attention for

malaria as often as permanent residents. As a consequence, nomads receive little or no residual house spraying and minimal chemotherapy. They serve as a mobile reservoir of malaria.

7. I suggest that DDT test papers be used in future WHO tests to determine when and if vector populations become susceptible again.
8. There is a dire need for faster turnaround time on positive malaria slides (cases) between tabulation and reporting information to Malaria Control Program people. There is currently a three-to-four-month lag period. As a consequence, a malaria epidemic could come and go before focal spraying could be done. A maximum of one-month turnaround of data should be the goal.
9. According to Baluchistan MCP officers, water wells dug in rock next to Iran (Chaghai, Noshki) are prolific *Anopheles* producers that are the sole cause of malaria in the area. I suggest that these be larvicided, either with temephos or methoprene (both have WHO approval for use in potable water). Small larvivorous fish might also be considered.
10. In-depth entomological and genetic studies should be planned and conducted to learn more about the malaria vectors in Pakistan. Some questions are these: Is *An. stephensi* a sibling species complex of more than two subspecies? Is vector potential and insecticide susceptibility different in the two subspecies? Is *An. culicifacies* a sibling species complex of more than four species? What is the importance of the other minor malaria vectors in malaria transmission, and are these species resistant to insecticides? Some of these minor vectors reported from Pakistan are *An. fluviatilis*, *An. sergenti* and *An. subpictus*. In the field collections I was involved in, *fluviatilis* and *subpictus* were collected. In fact, *subpictus* was more abundant than *stephensi* at one collection site.

11. Field trials in the different provinces under varying environmental conditions should be conducted on alternative insecticides, such as carbamates, pirimiphos methyl (OP), permethrin (pyrethroid) and others, to evaluate efficacy, residual life and applied costs. Such testing would prepare the MCP to switch to a new insecticide if malathion resistance becomes more widespread.
12. *An. culicifacies* has been found to be resistant to malathion in only two districts in the country (Vehari and Jhang, both in Punjab). Inasmuch as *culicifacies* has been selected equally by residual house spraying with malathion throughout the country, why hasn't similar resistance developed elsewhere? If one looks at Vehari, the explanation is probably in the agricultural spraying of cotton and other crops. I suspect that insecticide selection pressure occurred in the larval stage as a result of insecticide sprays hitting significant numbers of *culicifacies* breeding sites. Alternatively, agriculturally applied insecticides may be selecting both larvae and adults in the same generation. Resistance may have carried over into the adults and revealed itself in the WHO susceptibility tests. And I suspect the same scenario in Jhang. Apparently residual house spraying with malathion alone does not provide enough selection pressure, at least in the case of *An. culicifacies*, to produce resistance in adult females.
13. Public information and education on malaria prevention is lacking throughout the country. Printed information, such as posters and leaflets, and audiovisual information, such as public service announcements for radio and TV, explaining malaria prevention and control in their broadest context would be invaluable in enlisting public help and support. Such public education would go a long way in increasing the general public knowledge about malaria in the country. Information should be printed and recorded in all local languages and dialects.